myelin substrates (Figs. 2A-D), cells were fluorescently labeled with DiI, and plated on myelin (Fig. 2A), polylysine (Fig. 2B), or myelin +1 μg laminin (Figs. 2C and D). Control IgG was added to samples shown in Figs. 2A-C, the 3A3 antibody to the sample shown in Fig. 2D. Neurites do not extend on myelin but grow on laminin or mixed laminin/myelin substrates. When 3A3 is added, laminin no longer overrides growth inhibition by myelin. Figs. 2E-H show by phase contrast cells plated on recombinant MAG (Fig. 2E), laminin (Fig. 2F), or recombinant MAG plus laminin (Figs. 2G and H), with control antibody (Figs. 2E-G) or with 3A3 (Fig. 2H). Integrin function is needed to override growth inhibition by MAG.

Please replace the paragraph beginning at page 7, line 14, with the following rewritten paragraph:

--Fig. 3 presents the results of studies in which PC12 cells transfected with dominant negative Rho extend short neurites on MAG substrates. Mock-transfected PC12 cells (Figs. 3A, C, and E) or cells transfected with dominant-negative Rho (Figs. 3B, D, and F) were plated on laminin (Figs. 3A and B) or MAG (Figs. C-F). MAG inhibits neurite outgrowth (Fig. 3C), but dominant negative Rho cells spread on MAG and some cells extend short neurites (Fig. 3D). Treatment with C3 further stimulates neurite outgrowth on MAG from both lines of cells (Figs. 3E and F).--

Please replace the paragraph beginning at page 7, line 20, with the following rewritten paragraph:

--Fig. 4 shows activation of Rho on MAG substrates. Activated Rho is associated with the plasma membrane. To determine if activated Rho was detected under conditions where PC12 cells do not grow neurites, cells were grown in suspension or plated on MAG or collagen substrates. Two hours later the plasma membranes were purified, the proteins separated by SDS PAGE, and the proteins transferred to nitrocellulose and stained with Ponceau S (top panel). Rho A was detected on the blots by immunoreactivity with anti-RhoA antibody (bottom panel). Immunoreactivity was strongest when cells were grown in suspension or when cells were plated on MAG. Therefore, Rho A is more active when cells are kept in suspension or plated on MAG than when plated on growth-permissive collagen.--

Please replace the paragraph beginning at page 8, line 2, with the following rewritten paragraph:

E5

Fig. 5 shows treatment of retinal neurons with C3 stimulates neurite growth on polylysine and MAG substrates. On nMAG substrates neurite growth is inhibited (Fig. 5A), but after C3 treatment retinal neurons plated nMAG substrates extend neurites (Fig. 5B). Growth of neurites from retinal neurons plated on PLL (Fig. 5C). Bar, 50μm.

Please replace the paragraph beginning at page 8, line 6, with the following rewritten paragraph:

E6

Fig. 6 demonstrates ADP-ribosylation of Rho by C3 detected in cultured cells. PC12 cells or retinal neurons were cultured in the presence (+) or absence of C3 (-) for two days. The cells were lysed, and 10 μg of protein from each sample was separated on a 11% acrylamide gel. The proteins were transferred to nitrocellulose, probed with mouse anti-RhoA antibody and anti-mouse-HRP antibody, and revealed by a chemiluminescent reaction (top panel). The membranes were then reprobed with rabbit anti-Cdc42 and anti-rabbit alkaline phosphatase and revealed with NTB/BCIP color reaction (bottom panel). Treatment of cells with C3 resul[a]ts in an ADP-ribosylation-induced decrease in the mobility of RhoA. The mobility of Cdc42 does not change with C3 treatment.

Please replace the paragraph beginning at page 8, line 14, with the following rewritten paragraph:

E7

Fig. 7 illustrates methods used to study the effect of C3 on injured optic nerve. Figure 7a shows the optic nerve was removed from the sheath prior to crushing with 10.0 sutures (top panel) and C3 was applied in Gelfoam and Elvax tubes (rectangular bars in middle and bottom panels) immediately following optic nerve crush (middle panel). The retinal ganglion cell axons were detected by anterograde labeling with cholera toxin and immunodetection of the cholera toxin in longitudinal sections of the optic nerve (bottom panel). Figs. 7C-F show treatment of crushed optic nerve with C3 stimulates regenerative growth of retinal ganglion cells axons. (Fig. 7C) Longitudinal 15 μm section of a buffer-treated control optic nerve showing the failure of

RGC axons to cross the injured region; (Figs. 7D and E) Longitudinal 15 μm sections of two different optic nerves treated with C3 showing anterogradely-labeled axons extending past the crush (arrows). The site of crush is indicated with arrowheads; (Fig. 7F) Higher magnification view in Fig. 2E showing the twisted growth of regenerating axons. Bar, 100 μm (Figs. 7C-E) and 50 μm in Fig. 7F. Fig. 7B shows quantitation of axon regeneration across the site of lesion. Representation of regeneration observed in different animals. For each animal, the maximum number of axons observed in a single 14 μm section was counted at different distances from the site of the crush. Each point represents one animal, but animals with growth past 500 μm are also represented at the shorter distances. Large numbers of regenerating fibers (>10/section) were observed to cross the lesion after C3 treatment compared to treatment with PBS.

Please replace the paragraph beginning at page 27, line 11, with the following1 rewritten paragraph:

To test the involvement of Rho in the response of primary neurons to MAG and to myelin substrates, we purified retinal neurons and treated them with C3. Neurite outgrowth from these cells was inhibited by MAG (Fig. 5A). As with PC12 cells, treatment of retinal neurons cells with C3 allowed neurite extension on the growth inhibitory MAG substrates to an extent similar to that observed on control substrates (Figs. 5B and C).

Please replace the paragraph beginning at page 28, line 8, with the following rewritten paragraph:

To explore the possibility that treatment of damaged axons with C3 might foster regeneration *in vivo*, we examined regeneration of retinal ganglion cell (RGC) axons in the optic nerve 2 weeks after optic nerve crush. Recently, it has been shown that microlesions in the CNS reduce the extent of the glial scar and allow axons access to CNS white matter distal to the lesion (Davies, S.J.A., *et al.* (1997) *Nature* 390, 680-683). To make microlesions of optic nerve, 10.0 sutures were used to axotomize RGC axons by constriction (Fig. 7A). Retrograde labeling of RGCs from the superior colliculus (not shown), as well as anterograde labeling techniques (eg., Fig 7A) verified that RGC axons were effectively axotomized. To apply C3 to crushed nerves, Gelfoam soaked with 2 mg/ml C3 was wrapped around the left optic nerve at the crush site, and

two Elvax tubes, each loaded with 20 mg of C3, were positioned for sustained slow release (Fig. 7A). Twelve animals were treated with C3 and a further 8 animals were treated with PBS as controls. Crushed and regenerating axons were visualized by anterograde labeling with cholera toxin injected into the eye 12 days after optic nerve crush (Fig. 7A). Fourteen days after optic nerve crush, longitudinal cryostat sections of the optic nerves were examined by fluorescent microscopy for immunoreactivity to cholera toxin to detect anterogradely labeled RGC axons.

E9

Please replace the paragraph beginning at page 28, line 23, with the following rewritten paragraph:

In control optic nerves that received optic nerve crush alone, no RGC axons extended past the crush site (n= 3 animals). In control animals treated with PBS-Elvax pellets and gelfoam, the crush site was easily detected where most anterogradely labeled axons stopped abruptly (Fig. 7C). However, in these animals, a few axons did extend past the crush (Fig. 7C, arrows), and the numbers of axons that regenerated varied from animal to animal. The application of Gelfoam and Elvax tubes may have altered the response to injury. Nonetheless, the response to C3 treatment applied with this lesion paradigm was dramatic.

E10

Please replace the paragraph beginning at page 29, line 4, with the following rewritten paragraph:

We observed that C3 treatment allowed many RGC axons to grow past the region of the lesion. In 7 of 12 C3-treated animals, the lesion site was not clearly defined because of the large numbers of axons that extended through the site (Figs. 7D and E). Many of the axons that extended past the lesion site showed a twist path of growth, supporting their identification as regenerating axons (Fig. 7F) A quantitative comparison of C3 and PBS treated animals revealed that more fibers grew past the lesion site after C3 treatment than after PBS treatment (Fig. 7B). For this analysis we made a conservative estimate of the lesion site based on morphology, and counted the number of fibers in the distal optic nerve in 14 μm sections. Seven of 12 C3-treated animals showed at least one section with 10-20 axons extending 250 μm past the crush, compared with 1 of 8 of the PBS-treated controls (Fig. 7). In some animals regenerating axons were observed up to 1 mm from the crush, an extent of regeneration similar to that observed in

E11

mouse optic nerve after treatment with IN-1 antibody to block myelin inhibitors where fibers extended up to 750 μm ([(]Bartsch, U., *et al.*, (1995) *Neuron* 15, 1375-1381).--

Please replace the paragraph beginning at page 29, line 18, with the following rewritten paragraph:

--Rats were anesthetized with 0.6 ml/kg hypnorm, 2.5 mg/kg diazepan and 35 mg/kg ketamin. The left optic nerve was exposed by a supraorbital approach, the optic nerve sheath slit longitudinally, the optic nerve lifted out and crushed 1 mm from the globe by constriction with a 10.0 suture held for 60 seconds (Fig. 7A). For C3 treatment and buffer controls, Gelfoam soaked in PBS or 2mg/ml C3 transferase was placed on the nerve at the lesion site. Two 3 mm long tubes of Elvax (Sefton, *et al.*, (1984) loaded with buffer or 20 mg C3 were inserted in the Gelfoam near the nerve for continued slow release of C3 (Fig. 7A). Twelve days after crush, 5 ml of 1% cholera toxin β subunit (List Biological laboratories, Inc., Cambell, CA) was injected into the vitreous to anterogradely label retinal ganglion cell axons (Fig. 7A). Two weeks after optic nerve crush the animals were fixed by perfusion with 4% paraformaldehyde, and the eye with attached optic nerve was removed and postfixed in 4% paraformaldehyde. Longitudinal cryostat sections were processed for immunoreactivity to cholera toxin with goat anti-cholera toxin at 1:12,000 (List Biol. Labs Inc., CA) followed by rabbit anti-goat biotinylated antibody (1:200, Vector Labs, Burlingame, CA), and DTAF-streptavidin (1:500, Jackson Immunoresearch Laboratories).--

E12

In the claims:

Please cancel claims 32 and 33.

Please add claim 34.

In the abstract:

Please add the following abstract:

--The invention provides a method of suppressing the inhibition of neuronal axon growth. The method involves delivering *Clostridium botulinum* ADP-ribosyl transferase directly at a nervous system legion site in a patient.--

E13